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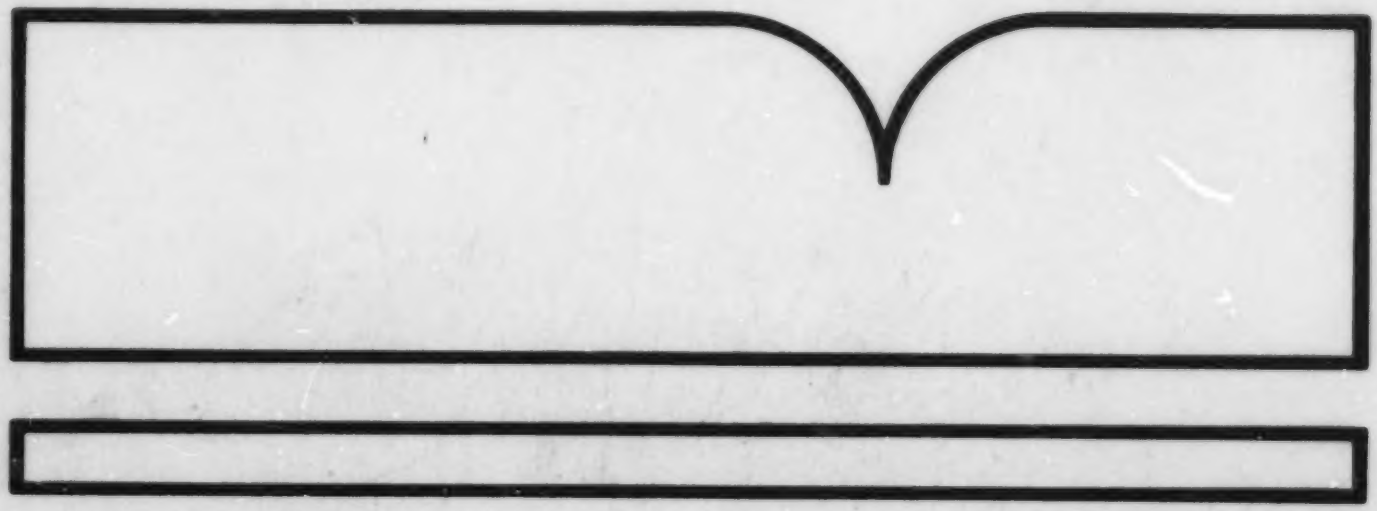

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16. ABSTRACT

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Characterization of Organics in Tannery Effluents by Liquid Chromatography Mass Spectrometry†

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Combined high performance liquid chromatography mass spectrometry using a moving belt interface has been used to study extracts from five tannery and leather finishing industry discharges. Liquid chromatographic separation was performed with a Zorbax CN column using a normal phase solvent system of hexane + methylene chloride + methanol. Twenty different compounds were identified. Binaphthyl sulfone, which could not be gas chromatographed successfully, was one of the major components found in all five tannery extracts.

INTRODUCTION

The analysis of industrial and environmental wastes for unknown organic pollutants is generally limited to those compounds that are volatile enough to pass through a gas chromatograph for identification by a mass spectrometer. However, many polar or heat labile organics are not directly amenable to gas chromatographic analysis; they are often overlooked or not measured because no practical methods of analysis have been available.

High performance liquid chromatography (LC) extends analytical capabilities by offering techniques to selectively separate samples containing volatiles, polar or heat labile organics. Liquid chromatography combined with mass spectrometry (LCMS) provides a separation technique and a detector that can identify many chemical structures. Presently mass spectrometric analyses of LC peaks are usually performed off-line by collecting individual LC peaks, evaporating solvents, and loading solutes into the mass spectrometer via direct probe.[1,2] This is time-consuming and tedious, and small unresolved peaks are not always easily collected. Although there are several methods for direct coupling of a high performance liquid chromatograph to a mass spectrometer, at the present time there are only two LCMS interface designs commercially available. One of these is based on introduction of a very small fraction of the LC effluent into the mass spectrometer (using the solvent as reagent gas for chemical ionization) and one uses a moving belt transport in a vacuum lock.[3,4] Each has advantages and disadvantages depending on the sample. The former is inexpensive but gives chemical ionization (CI) spectra only; the latter allows choice of electron impact (EI) or chemical ionization mode of mass spectrometric operation.

The research presented here shows the utility of using the moving belt system for identifying the components of complex mixtures typical of environmental extracts.

† Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the US Environmental Protection Agency.

EXPERIMENTAL

Sample background

Tannery and leather finishing industry discharges from various plants located in the northeastern United States were surveyed in 1978 to characterize pollutants in tannery effluents. This survey was part of the Environmental Protection Agency's commitment to establish effluent guidelines for selected organic compounds from major industries. These samples were analyzed by an EPA screening procedure which serves as a general method for analytical surveys, in which all volatile organic compounds above a specified level in water are searched for and quantified.[5] This method consists of extracting a 1 l portion of wastewater with 200 ml of methylene choride, concentrating the extract to 1 ml, and analyzing the extract by GCMS.

The volatile compounds amenable to gas chromatography (GC), reported by the EPA contractor to be in these extracts included chlorinated benzenes, polycyclic aromatics, phthalates, phenol, and chlorinated phenols. After the EPA contractors completed GCMS analysis, five of the tanning industry extracts were analyzed by LCMS at the Athens Environmental Research Laboratory.

Instrumentation

Liquid chromatographic separations were performed on a Waters Associates 204 liquid chromatograph equipped with two Model 6000 pumps, a Model 660 solvent programmer, and a Model 400 dual ultraviolet (UV) absorbance detector (254 nm and 280 nm). The column used was a 4.6 mm × 25 cm Zorbax CN. Flow rate was 1.6 ml min^{-1}, using a gradient of hexane to methylene chloride + methanol. The LC effluent from the absorbance detector was coupled to a 1 : 1 tee that split 0.8 ml to waste and fed 0.8 ml to a Kapton-belt-transport Finnigan LCMS interface.[4] The belt introduced the sample into a Finnigan 3200 mass spectrometer used

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either in the EI or the methane CI mode at 70 and 100 eV, respectively. The initial source temperature was 60 °C; the interface vaporizer was 170 °C. The emission current was 0.5 ma. The mass spectrometer was interfaced to a System 150 data system.

Solvents

The solvents were 'Distilled in Glass' UV grade *n*-hexane, methylene chloride, and methanol from Burdick and Jackson Co., Muskegan, Michigan, USA.

Liquid chromatograph mass spectrometer operation

The Finnigan LCMS interface conducts the LC eluant from the absorbance detector directly onto a continuously moving belt. The solvent is partially evaporated under an infrared heater and then almost entirely removed through two differentially pumped vacuum locks before being introduced into the ion source.

Operating pressure in the mass spectrometer source is generally 10^{-5} to 10^{-6} Torr. The solvent flow rate that can be accepted by the interface depends on the solvent volatility. Too high a flow rate onto the belt results in incomplete evaporation of the solvent by the infrared heater or the vacuum locks. This causes erratic vacuum fluctuations between 10^{-5} and 10^{-4} Torr that obscure mass spectrometric data. Volatile nonpolar solvents such as pentane or hexane are usable at flow rates up to 1.5 ml min^{-1}; flow rates for intermediate polarity solvents such as methylene chloride and chloroform can reach about 0.5 ml min^{-1}. Polar solvents such as methanol and isopropanol are usable at 0.2 ml min^{-1}; water can be used at about 0.1 ml min^{-1}. Flow rates during a gradient run are compromises of these flow rates.

Environmental sample conditions

Environmental samples contain compounds having a wide range of polarities. Earlier studies in this laboratory[6] showed that a silica column, using a solvent gradient of hexane to chloroform or alcohol, can separate compounds from environmental extracts into several polarity ranges. Eluting with the solvent front are such low polarity compounds as polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, and aliphatic hydrocarbons. As the polarity of the solvent increases, more polar compounds such as quinones and phthalates elute.

In the present investigations of tannery effluents, it was found that a bonded silica column, Zorbax CN, gave good resolution for separation of these complex extracts. A 30 min linear gradient of 100% solvent A (4% methylene chloride in hexane) to 60% solvent B (2% methanol in methylene chloride) at a flow rate of 1.6 ml min^{-1} was optimum for good separation on the column. The solvent effluent exiting from the absorbance detector was split by a tee that directed a 0.8 ml min^{-1} flow directly onto the LCMS interface belt. The gradient rate, percentage of alcohol in methylene chloride (6% isopropanol in methylene chloride gives almost the same retention times), and flow rate can be adjusted slightly to change retention times of overlapping peaks; the effluent split can also be changed, but generally a 0.5 to 1.0 ml min^{-1} flow on the interface belt is optimum.

The mass spectrometer time should be set less than the expected LC peak width—around 5 s per scan. A 15 s LC peak will generally give good mass spectrometric data for study of an unknown mixture. Depending on the compound, it is possible to obtain full spectra scans (90–400 amu) on 100–1000 ng quantities.

For different compounds, depending on their vapor pressure, varying relative amounts are transported by the LCMS belt into the mass spectrometer source. Although McFadden[4] reports 30–40% efficiency for transfer of most compounds, transfer of organics typical of the tannery industry has not been evaluated. Because of this uncertainty, no attempt at quantitation was made in the work described here.

Procedure

The methylene chloride extract (50 ul) is injected onto the LC column and the gradient program started. After about 3–4 min, when the solvent peak containing aliphatic hydrocarbons and nonpolar aromatics has passed, the data acquisition is initiated. Because small traces of residual solvent are always present (e.g. methylene chloride's last appreciable mass at m/z 88), the lowest mass that can be scanned is around m/z 90. Many low molecular weight compounds are difficult to identify due to the absence of this very important mass spectral region below m/z 90.

RESULTS AND DISCUSSION

Tannery extracts

Figure 1 compares LC chromatograms of a typical tannery effluent for three different detectors. The UV detector is quite useful for working out LCMS procedures. The UV chromatogram and the EI or CI total ion current (TIC) could be matched well when allowance was made for a 30 s time delay between the UV peak and the peak appearance indicated by the TIC. All identified compounds absorbed at either the 254 nm or the 280 nm wavelength with the exception of cholesterol. It was detected only by the LCMS.

The methane CI mode produced a higher baseline noise level than the EI mode due mainly to higher pressure in the source. CI mass spectra compared favorably with standard methane CI spectra. (No interaction occurred from residual solvents or excess air or water.)

Table 1 shows the compounds found by LCMS in five different tanning industries. Results show many compounds are common to the different tanning plants. The compounds were identified by comparing mass spectra to reference spectra and, in general, each compound was confirmed by comparing liquid chromatographic retention times and mass spectra with

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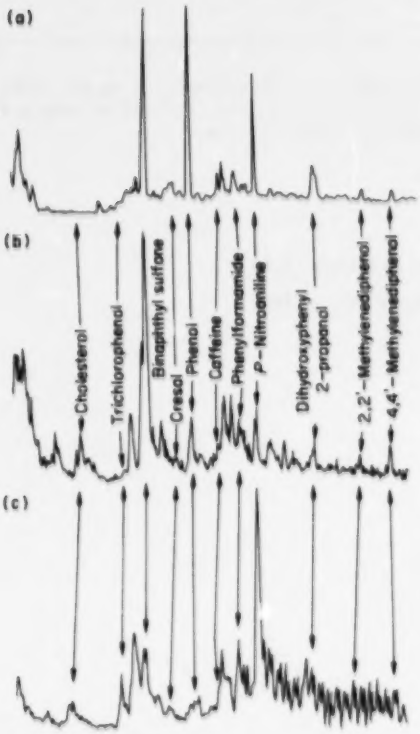

Figure 1. High performance liquid chromatography chromatograms of a tannery extract. Column is Zorbax CN with a 30 min linear gradient of 100% solvent A (4% methylene chloride in hexane) to 60% solvent B (2% methanol in methylene chloride). (a) UV 254 nm; (b) EI total ion current; (c) CI total ion current.

Table 1. Organics found in five different tanning industries

	Tanning industries sampled				
	D	B	S	M	C
Phenol	•	•	•	•	•
Binaphthyl sulfone	•	•	•	•	•
Cholesterol	•	•	•	•	•
p-Nitroaniline	•	•			•
p-Cresol	•	•	•	•	
o-Cresol	•				
Caffeine			•		
o-Phenyl phenol	•		•		
2,4,6-Trichlorophenol	•		•		•
Pentachlorophenol	•		•		•
2,2'-Methylenediphenol	•	•	•	•	•
4,4'-Methylenediphenol	•	•	•	•	•
2,4'-Methylenediphenol	•				
2-Naphthol	•			•	
4-Chloro-2-methylphenol	•				
p-Nitrophenol		•			
N-Phenylformamide		•			
3,5,7-trihydroxycoumarin[a]			•		•
n-Propylthiophene[a]					•
2,5-dihydroxyphenyl-2-propanol[a]		•			

[a] Tentative identification.

standards. No standards were available for 3,5,7-trihydroxycoumarin, *n*-propylthiophene and 2,5-dihydroxyphenyl-2-propanol. They were tentatively identified by comparing with data base reference spectra only.

Binaphthyl sulfone

Binaphthyl sulfone is one of the major components in all five tannery extracts. It was tentatively identified by mass spectral fragmentation patterns. The mass spectrum was absent from available mass spectral matching tables or data bases and no standard was available, so the compound was synthesized.[7] Sulfuric acid was reacted with naphthalene, and the reaction mixture was extracted with solvent. The resultant extract was analyzed by LCMS without additional clean-up. The liquid chromatographic retention time, UV responses, and mass spectrum matched those of the sample extract. Three isomers are possible for binaphthyl sulfone—the σ,α'-, the β,β'- and the α,β'-. No attempt was made to identify the specific isomer found in these samples.

The EI mass spectrum of binaphthyl sulfone is shown in Fig. 2. The ion at m/z 318 is the molecular ion and methane CI confirms this molecular weight. The abundant fragment ion at m/z 253 is formed by a loss of 65 amu (SO_2H) from the molecular ion, while the abundant ion at m/z 127 is due to the loss of 191 amu ($SO_2C_{10}H_7$) from the molecular ion and probably arises from cleavage of both C—S bonds. Under the less energetic CI conditions, a single C—S bond is broken showing a large m/z 191 ion due to the loss of 127 amu ($C_{10}H_7$). The abundant ion at m/z 175 is apparently the result of sulfur–oxygen rearrangement causing a loss of 143 amu ($OC_{10}H_7$).

Binaphthyl sulfone could not be gas chromatographed successfully and has never been identified in environmental samples by GCMS or direct LCMS. The compound has been found previously in river water extracts by trapping of an eluted LC fraction, followed by probe mass spectrometry.[1,2]

In summary, environmental samples contain complex mixtures of volatile and nonvolatile organic compounds covering a wide polarity range. LCMS shows promise for analyzing environmental samples and detecting and identifying compounds not amenable to conventional GCMS analysis.

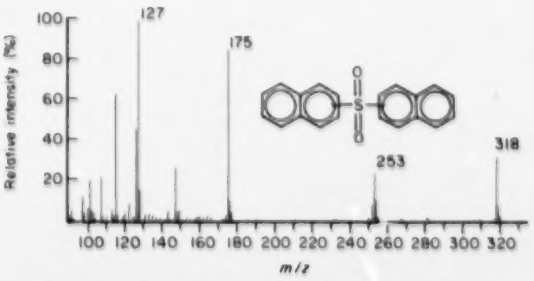

Figure 2. EI mass spectrum of binaphthyl sulfone.

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